Propagation Handbook for the Karner Blue Butterfly, *Lycaeides melissa samuelis*

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Introduction

The object of this propagation manual is the Endangered Karner blue butterfly (KBB), *Lycaeides melissa samuelis*, which has suffered catastrophic population declines in the U.S. over the past decade. The KBB has declined by 99% or greater over its range in the past 100 years (USFWS 1992), and an IUCN PHVA (IUCN 1992) estimated that 90% of this decline has occurred in the past 10-15 years. Within this period the KBB has been extirpated from Illinois, Massachusetts, Ohio, Ontario, and Pennsylvania. As our current KBB research season was nearing an end, we learned that the New Hampshire population was also extirpated. The KBB is a signature species of the extremely rare oak savanna ecosystem, a globally endangered habitat characterized by meadows of prairie plants dispersed among stands of widely spaced oak trees. Oak savanna in Ohio is limited to a small region of Northwest Ohio known as the Oak Openings. It was the last area in Ohio where the KBB was observed (Magdich 1989; Grigore and Windus 1994). The region, as well as the KBB, has national significance, and the dedication to restore the KBB to Ohio is shared by a powerful coalition of conservation partners in this project, which includes the U.S. Fish and Wildlife Service (USFWS), the Ohio Department of Natural Resources (ODNR), and The Nature Conservancy.

This handbook is not meant to be the last word in Karner blue butterfly propagation. Each season is a learning experience for us, and we constantly strive to apply results of our current research to KBB conservation breeding. We still have problems relating to low fertility in certain egg clutches, and questions remain about the factors contributing to fertility in captivity for this species.). However, it is both possible and practical to mass-rear hundreds of KBBs for release or conservation breeding, housing pairs or groups of reproductive butterflies or larvae in individual pots containing living lupine and nectar plants. These techniques were developed in consultation with the USFWS and ODNR KBB Recovery Teams. There have been some excellent KBB rearing studies completed in the past and the reader is encouraged to consult them (See Lane and Welch 1993; Herms et al. 1996)

Propagation of host plant- *Lupinus perennis*

Local lupine seed is collected in July before dehiscence is complete. Seed is stored dry outdoors until the following spring. Immediately before planting seeds are scarified with medium grade sandpaper and are placed in hot water for 1 hour. Seeds are strained from the water and mixed with *Rhizobium* inoculate, type H (Prairie Moon Nursery, Winona, MN) and placed in a clear plastic box for 24 hr. in sunlight. The next day, seeds are planted in 2-gallon plastic pots in the following sand mix:

- 3-4 parts sand
- 1 part potting soil
- 1 part sphagnum peat
- 1 part small pine bark chips
- 1 part perlite

An alternate potting mix has been suggested by the Portage Valley Nursery in Holland, Ohio, consisting of:

- 1 part sphagnum peat
- 1 part small pine bark chips

Wild lupine is extremely susceptible to root rot, especially in the winter, and any soil mix must be well draining.

Five seeds are planted per 2- gallon pot at a soil depth of 1/4 inch. Seeds are watered thoroughly every day in summer; once a week in the winter months. Plants are maintained under shaded poly in open air, and are fertilized every week throughout the growing season to increase protein content for larvae. Some recent research has also suggested that protein deficiency reduces lifespan in certain pollen and nectar feeding insects (Schmidt 1998) and that fecundity in some lepidoptera may be limited by protein obtained as larvae (Stamp et al. 1993) or adults (Labine, 1986; Pierce 1985; Dunlap-Pianka 1995). Spider mites commonly infest these plants. In the event of spider mite or white fly infestation the foliage is cleansed with a solution of dish soap (1/4 tsp./cup) and water applied with a spray bottle. The treated plants should be set aside for several days and then thoroughly rinsed with tap water before allowing them to be used by larvae.

Collection

The Karner blue 1st brood flight period usually begins the second week in May at our latitude and lasts for three weeks. We consult with the Michigan Department of Natural Resources staff biologists at the Allegan State Game Area in early May to learn when the flight has commenced. Our first collection begins on a morning one-week after the first KBB sighting in the spring. Then a collection date is scheduled one morning each week after that until the adequate number of females has been obtained. A collection of wild caught females is assembled from several different locations to insure genetic diversity. The females also collected on different dates, spaced approximately one week apart to insure that the majority of the females collected were recently emerged and gravid. Our greatest egg production has always been from females collected during the second week of the flight. The females are captured with a large butterfly net and transferred to a 10 cm x 10 cm x 18 cm transparent, plastic container (AMAC Plastics Products Corp., Sausalito, CA) marked with the date and capture locality. An artificial nectar source is fastened inside of each plastic container consisting of a rubber-capped florist tube with a cotton wick protruding through the cap filled with a 10% clover honey/water solution. This allows the butterflies to have access to some nourishment and prevents dehydration during the transport. This is especially important, as recently emerged females may not have had a chance to feed.

Transport

The plastic containers are placed within thick-walled 32 quart Styrofoam coolers that contain a single ice pack separated from the butterfly containers by bubble-pack insulation. The temperature inside the cooler was maintained at <20° C (because of the chill and darkness the butterflies are less active and therefore less likely to batter themselves en route). A data logger is placed inside of the cooler to record the temperature. A digital electronic thermometer with a probe is used to monitor the cooler temperature in real time. Readings are taken every 30 minutes. Following collection and storage the butterflies are immediately brought back to the holding area at the zoo. We have maintained a 100% survival rate for collection and transport using this method.

Holding and care of adults

Upon arrival at the Zoo the butterflies are placed into an enclosure consisting of a mesh- covered potted lupine plant in a 2-gallon plastic pot. This covering is a cylinder of white poly mesh netting # 65-50 (Jason Mills, Westwood, NJ) sewn together with the seams arranged on the outside of the enclosure to assure that none of the butterflies can become trapped in the seam and harm themselves. Lycaenids tend to walk into corners and crevices and may become trapped. The nets fit snuggly over the pot rim, and are secured with a # 107 (7” x 5/8” x 1/16”) rubber band to prevent escape of butterflies and to deny entrance to predators. Each pot is numbered with a stamped metal tag identifying each butterfly with a studbook number. Each mesh-covered lupine plant contains a small 3" pot of *Lantana camara* or *Pentas sp.* as a nectar source. These plants were chosen because of their short flower tubules, for their long blooming period, and availability. We normally purchase the nectar plants from commercial nurseries. It is extremely important that the plants be pesticide-free before exposing butterflies to them. An additional nectar source is provided from a rubber-capped florist tube with a cotton wick protruding through the cap filled with a 10% clover honey/water solution (the same unit used as artificial nectar source in transport). This is placed in the soil of the potted plant. The artificial nectar source is refilled every day and replaced and disinfected in diluted common household bleach every other day. Disinfected tubes and wicks are rinsed 10x in tap water and 5x in distilled water before being reused. We recommend daily hand feeding of adults to maximize longevity. In this process, adults are encouraged to climb on the honey-moistened wicks of the feeing tubes. They are then gently placed with the tubes in one of the10 cm x 10 cm x 18 cm transparent plastic containers use for transport. After the proboscis is withdrawn, the butterflies are placed back into their netted enclosures. For breeding males, a slurry of animal dung should be provided to help provide essential nutrients for the sperm packet.

Wild caught females are monitored every day for egg laying. An official egg count is performed every 2nd day. Eggs are counted by visually inspecting each leaflet and stem of the lupine plant. The undersides of the leaflets are viewed with a dentist mirror. One must make sure that the nectaring plants are adequately watered to insure that they are producing nectar. The condition of the host plant and nectar plant is also monitored every other day. We have estimated the ideal carrying capacity of each healthy host plant to be between 10-12 larvae/lupine. When 10 eggs have been laid the female is moved to a new host plant. However, some females may lay over 50 eggs in a single night. Nectar plants that are replaced when the flower heads are spent should be also checked for eggs. We prefer to remove eggs by simply clipping off the area of foliage containing the egg/s and placing it in the host plant pot. In the event that a host plant should become infected with spider mites, the foliage supporting eggs can be gently washed, clipped from the infected plant, and transferred to a healthy host plant. Loose eggs, such as those oviposited on the substrate, can be gently transferred with a damp #2 camel hair artist brush.

It is important to maintain humidity in the enclosures. To prevent dehydration the mesh-covered enclosures are misted by hand or, during periods of heat and low humidity, by an Ecologic Technologies® Rainmaker misting system (Ecologic Technologies, Pasadena, MD). The Rainmaker was set up to mist for a 2-minute duration every 15 min. from 1100-1800 h. In extremely hot weather (> 30^{o} C) a garden soaker hose is placed on the cement pad near the enclosures to provide added humidity through evaporation.
Cool temperatures and low light intensity may prevent females from oviposition. The enclosures are arranged on shelves in an area with high light intensity and on overcast days quartz lighting was provided. In years when there are low temperatures in late May and early June a large propane heater is also employed to warm the polyhouse.

Inventory and holding of larvae/pupae

The wild-caught females are assigned a house studbook number maintained in an informal Microsoft Excel© studbook with such information as their capture location, capture date and fecundity. A running total is kept for all eggs, larvae, pupae, and adults produced by a given female in the studbook.

In addition to the studbook, a data sheet is produced for each female each time the eggs, larvae, pupae, or 2nd flight adults are counted. Examples of a studbook page and the data sheets are provided in Appendix A.

A numbered metal tag is attached to the pot of the first mesh-covered lupine plant used. When that lupine plant is replaced for a given female, a secondary number is assigned to the next pot to identify the founder of each individual set of eggs. For example, the first pot for female # 45 would have a numbered metal tag reading "45". After 10-12 eggs/lupine have been laid then the next pot used for that female would be affixed with a plastic tag reading "45A", then, for the next "45B", and so on. This enables us to easily keep individualized founder information on each set of eggs. This also allows us to look back and attempt to determine why a certain set of eggs did not hatch, or why certain pupae lived and others, in a different pot from the same female, did not. See Table I.

Studbook number	Capture date	Death date	Locality	Pot or Enclosure	Number of eggs	Number of larvae	Number of pupae	Number of adults	As of
116	22-May	13-Jun	Pipeline	116, A, B, C	6	0	0	0	25-May
				D	14	0	0	0	28-May
					15	0	0	0	30-May
					16	0	0	0	1-Jun
					19	1	0	0	4-Jun
					24	9	0	0	5-Jun
					27	10	0	0	6-Jun
					35	13	0	0	8-Jun
					41	15	0	0	11-Jun
					42	30	0	0	13-Jun
					57	36	0	0	15-Jun
					57	36	1	0	20-Jun
					57	43	5	0	22-Jun
					57	47	13	0	25-Jun
					57	47	17	0	27-Jun
					57	47	23	0	29-Jun
					57	47	27	8.1	2-Jul
					57	47	29	9.5	4-Jul
					57	47	30	13.7	6-Jul
					57	47	32	14.8	9-Jul
					57	47	32	15.14	11-Jul
					57	47	32	15.15	13-Jul
					57	47	32	15.16	16-Jul
					57	48	32	15.17	17-Jul

Table I. Informal studbook for 1st flight female Karner blue butterfies.

The larvae and pupae in each pot are counted and recorded three times a week on the data sheet and a running total is kept for each instar. Condition of larvae and pupae are noted. For example, pupae are green as pupation occurs, transform to a brown color as they age, and finally become semi-transparent when exclosure is imminent. At this time, the adult coloration, particularly the purple hues of the male, are visible through the semi-transparent cuticle of the pupa.

Larval counts will often fluctuate from day to day, as larvae will disappear, then reappear. They often move in and out of the soil around the root system and between the pot wall and the soil. Larvae are transferred to new host plants as leaves are denuded. It is important not to overcrowd the larvae or to keep larvae of differing sizes on the same host plant. Larger larvae can and will cannibalize their smaller conspecifics. As the larvae approach the 3rd and 4th instars they tend to become more mobile and will often crawl off the plant. It appears that in the fourth instar the innate behavior to find a perfect place to pupate will drive them to migrate more than usual. In captivity, they can often be found in dangerous places like the outside pot edge, under the pot, up in the net, or on the substrate near the pot. This makes it extremely easy to inadvertently smash the larvae or pupae. For this reason, extra care should be taken during the 4th instars when moving or handling the netted lupine enclosures. When pupation is imminent, pots are provided with several large pine bark chips. Fourth instar larvae often will pupate under these refugia. As soon as 2nd brood adults emerge they are sexed and recorded in the studbook. However, 2nd flight adults are not assigned their own studbook number- they are only identified by their mother's studbook number and locality. A running total of the 2nd flight adults is also kept under the studbook entry for the mother.

Predator control

Predators threaten KBBs in every stage of the life cycle. Spiders are the primary threats to larvae, pupae, and adults. Centipedes, which live in the soil, are a secondary threat. Ants, particularly the tiny *Monomormium emargeratum*, will predate KBB eggs. They may actually cut the egg almost in half, or cut a hole in the egg through which they pull the larva. They are most active in the morning and in the evening. Although *Myrmica sp.* ants protect KBB larvae in the wild, they will also predate the eggs. Trichogrammid wasps parasitize KBB eggs. Up to 7 wasp larvae may be deposited in each egg (Spoor, pers. comm.).

No pesticides are ever used in the polyhouse or on the host or nectar plants. We depend on vigilance and exclusion tactics to protect our KBBs. Each lupine plant must be thoroughly checked (i.e. examination of each leaflet) for predators before placing a KBB of any life stage upon it. Polyester netting covering the plants excludes predators, but each plant is checked every 2-3 days to ensure that no predators have entered the pots or have hatched from undetected eggs. Any detected predators are simply crushed with our fingers.

Adult care and breeding

Second brood adults are placed into breeding enclosures that are essentially individual camping tents with “no see-um” mesh. (Tropic Screen II®, Bioquip Products, Inc., Gardena, CA). These mesh tents have a nylon floor and are placed approximately 4" off of the cement pad of the polyhouse by plastic shipping pallets to keep them dry and allow ventilation. This prevents the formation of dangerous pools of water (that will catch and drown small butterflies) or harmful mildew growth. The parabolic shape of the tent prevents the adults from getting caught in corners or on the peak as they would with a conventional tent. The breeding enclosure (mesh tent) is equipped with six large, healthy lupine plants, six large *Lantana camara*, *Pentas sp.* or *Asclepias tuberosa* plants, and two large artificial nectar sources placed on pedestals. These are fashioned from disinfected 4" diameter white scrub pads cut to fit into 4" diameter Petri dishes and then saturated with the 10% clover honey/water solution. Elevating the artificial nectar source on a pedestal prevents the attraction of harmful pests like ants to the breeding enclosure and makes them more visible to the butterflies.

Males and females from the same locality (10.10) are placed within the breeding enclosures for mating and oviposition. Under this system females have the opportunity to serially mate. Serial mating may be important in this species, as we have seen egg fertility decline in subsequent clutches of wild-mated females (See Drummond 1984; Oberhauser 1974; Rutkowski et al 1997; Wannatabe 1988).

The adults are provided with a timed overhead misting system that was previously described under "holding and care of adults”. Dead adults are removed from the floor of the tent on a daily basis and replaced by new butterflies. It is difficult to count eggs oviposited in the tents because of the potential harm to the breeding adults. Oviposition is estimated by viewing the host plants through the mesh and determining whether or not adequate numbers of eggs have been laid. After the target numbers of eggs are reached for each locality (usually 50 eggs), the remaining 2nd brood adults are released at the reintroduction site. Deaths and daily releases of 2nd brood adults are recorded on a daily basis and tracked on a separate chart and a tag on each tent.

In the 2001 field season we discovered that adult longevity was significantly increased when the adults were hand fed a 10% clover honey solution daily. Butterflies are encouraged, by gentle nudging, to walk on the saturated wick of one of our artificial nectar tubes. They usually will immediately commence feeding. Using this technique, we have increased adult survival to as much as four weeks in the breeding facility.

2nd flight egg over-wintering protocol

Data collected from the field at the Allegan State Game area indicates that over-wintering eggs in the duff are subjected to very high humidity in the winter, usually 98-100% in a near- condensing atmosphere. Relative Humidity (RH) with infrequent spikes

to lows of 80 % RH. Temperatures under snow cover are remarkably stable- 0^{o} C to -5^{o} C. Our best 2nd brood hatching success (ca. 40% hatching) has occurred under these conditions. We place our eggs in Mason jars containing a 2" diameter insert constructed from Plexiglas tubing. The tubing has a support of chiffon fabric 1" below the lip of the tubing. To construct this support, a 9 " section of tubing is sawn into two pieces- one of 1" length, and one of 8" length. A circular patch of fabric is glued between these two pieces of tube using Silastic aquarium cement. Replacing the dome lid with a chiffon fabric insert glued into the band with silicone aquarium cement further alters the Mason jar and protects the eggs from marauding ants. Ten to 12 eggs are placed in each jar and the jar is labeled with the number of eggs and the collection locality of the parents. Eggs are whitish green when deposited but change color to a dirty gray as they over-winter. This color change is normal. In the extremely hot and dry months of July and August, ca. 200 ml of distilled water is added to the bottom of each jar to keep humidity levels high for the eggs. Care must be taken to ensure that no water condenses around the eggs as the chiffon fabric insert in the band may inhibit evaporation, particularly under rainy conditions when ambient RH is high. Lids can be removed from jars with condensation to allow condensed water to evaporate. Jars should never be left without tops overnight, as the eggs may be predated or parasitized. Jars can be placed in a shaded location protected from rain. We prefer to bury the jars, protected by covering them with a sheet of polyfilm, beneath snow cover when possible.

Release protocol

Second brood adults that have previously been in breeding enclosures or are freshly eclosed are transported to the reintroduction site in a mesh-covered pot containing lupine and a nectar plant. They are released in early afternoon in fair to good weather conditions in an area where there are adequate nectar and host plants.

Evaluation of release sites

Site selection-sites on KTP were chosen to represent a spectrum of lupine habitat, including mature oak savanna (Oak Dune, Julia's Savanna), lupine partially shaded by secondary forest (Bond Tract) and lupine in full sun on essentially treeless dunes (North Piels, South Piels). These sites included one which formerly supported populations of the Karner blue (Bond Tract), two which were not known to have KBBs but which support populations of the frosted elfin, *Callophrys irus*, and the Persius dusky wing, *Erynnis persius* (Oak Dune, Julia's Savanna), and two which have extensive patches of lupine but which do not support populations of any lupine-dependent butterflies (North Piels, South Piels). Data was collected concurrently in the Allegan State Game Area, Allegan County, Michigan, at three sites that support reproducing populations of the KBB (42nd St., Gun Club, and Pipeline). Sites were chosen in the Allegan State Game

Area based on their similarity in vegetation structure and species composition to those sites on the Kitty Todd Preserve that once supported populations of the KBB.

Methodology- percent canopy cover and density, frequency, and phenology of lupine and nectar plants were quantified at the sites described above. These data were collected in randomly selected 0.5m^2 quadrats using the transect-quadrat method of Bonham (1989) at the density of one transect / 850m^2 (Papp 1996). Density of lupine was calculated as the number of lupine stems/ m^2 within each quadrat. Brood 1 and brood 2 nectar plant densities were calculated as the number of flowering stems of nectar plants / m^2 within each quadrat (see Tables II and III for species lists of first and second brood nectar plants at KTP and the ASGA). Canopy cover was calculated using a crown densiometer. The number of hits per transect (one reading for each quadrat) were divided by the total number of quadrats at the site to give the percentage of canopy cover (Tolson 1998).

Species	Common name	Present at ASGA	Present at KTP	Exotic
Amelanchier spicata	Dwarf serviceberry	?	Yes	No
Arabis lyrata	Lyre-leaved rock cress	Yes	Yes	No
Arenaria stricta	Rock sandwort	Yes	?	No
Berteroa incana	Hoary alyssum	Yes	?	No
Fragaria virginiana	Wild strawberry	Yes	Yes	No
Geranium maculatum	Wild geranium	?	Yes	No
Helianthemum canadense	Frostweed	Yes	Yes	No
Hieraceum pillosella	Mouse-ear hawkweed	Yes	No	No
Krigia virginica	Dwarf dandelion	Yes	Yes	No
Lithospermum canescens	Hoary puccoon	?	Yes	No
Lithospermum caroliniense	Golden puccoon	Yes	Yes	No
Lupinus perennis	Wild lupine	Yes	Yes	No
Potentilla sp.	Cinquefoil sp.	Yes	Yes	No
Rosa carolina	Rose	Yes	Yes	Yes
Rubus sp.	Dewberry	Yes	Yes	No
Tephrosa virginiana	Goat's rue	Yes	Yes	No
Viola pedata	Birdfoot violet	Yes	Yes	No

Table II. Inventory of brood 1 nectar plants at ASGA and KTP utilized by the Karner blue butterfly.

Species	Common name	Present at ASGA	Present at KTP	Exotic
Achillea millefolium	Yarrow	Yes	Yes	Yes
Asclepias tuberosa	Butterfly milkweed	Yes	Yes	No
Asclepias verticillata	Whorled milkweed	Yes	Yes	No
Baptisia tintoria	Yellow indigo	Yes	Yes	No
Ceanothus americanus	New Jersey tea	Yes	Yes	No
Centaurea maculosa	Spotted knapweed	Yes	No	Yes
Coreopsis lanceolata	Lance-leaved coreopsis	Yes	Yes	No
Dianthus armeria.	Deptford pink	Yes	Yes	Yes
Erigeron annuus	Daisy fleabane	Yes	Yes	Yes
Euphorbia corollata	Flowering spurge	Yes	Yes	No
Helianthus divaricatus	Woodland sunflower	Yes	Yes	No
Helianthus occidentalis	Western sunflower	Yes	Yes	No
Hieraceum aurantiacum	Orange hawkweed	Yes	No	Yes
Hieraceum pratense	Yellow hawkweed	Yes	No	Yes
Hypericum perforatum	St. Johnswort	Yes	Yes	Yes
Lespedeza sp.	Bush clover	Yes	Yes	No
Liatris aspera	Blazing-star	No	Yes	No
Liatris cylindracea	Cylindric blazing-star	Yes	No	No
Lotus corniculatus	Birdsfoot trefoil	Yes	?	No
Monarda fistulosa	Wild bergemot	Yes	Yes	No
Monarda punctata	Dotted horsemint	Yes	Yes	Yes
Polygala polygama	Racemed milkwort	Yes	Yes	No
Rudbeckia hirta	Black-eyed Susan	Yes	Yes	No
Solidago sp.	Goldenrod	Yes	Yes	No
Specularia perfoliata	Venus looking-glass	Yes	?	Yes
Vicia cracca	Cow vetch	Yes	?	Yes

Table III. Inventory of brood 2 nectar plants at ASGA and KTP utilized by the Karner blue butterfly.

Remote computerized data loggers were programmed to take data readings every 30 minutes for surface temperature, solar radiation, and relative humidity at the level of the lupine crowns (i.e. ca. 5.0 cm above soil surface). Equipment used was Onset Computer Corp.'s StowAway temperature logger model STIB-37+46, StowAway relative humidity logger model SRHA-32, and Hobo light intensity logger model HLI. Each site had one each of the listed data loggers. Data were downloaded weekly, using the Onset Computer Corp. Stowaway and Logbook software, to a laptop computer. Percent canopy cover of oak and invasive secondary forest was also quantified at each site. Canopy readings were taken weekly over the season of activity for the KBB (normally May-July) as the canopy emerged.

Analysis- Data on nectar plant resource availability were recorded and analyzed separately for first and second brood KBBs. Comparisons between locations (Michigan and Ohio) were made using an independent measures t-test and analysis of variance (ANOVA). Tests were performed to determine differences among sites within each location. ANOVA was followed up with the Tukey test to determine which sites within ASGS and KTP varied from each other. The two highest potential reintroduction sites on KTP, i.e. those with the highest mean densities of lupine and nectar plants (Bond Tract, Julia's Savanna), were compared with each of the ASGA sites independently using an independent measures t-test.

Monitoring

Monitoring for 1st flight KBBs begins approximately the third week in May. The transect area is searched each day except in the rain until no butterflies have been sighted for at least three days. It is then suspended until the 2nd brood adults emerge, approximately 28 days after the 1st flight. Then monitoring resumes again in the same fashion until three days after the last 2nd flight KBB has been seen. Emigration from the release site is determined by carefully walking a transect route for each site in our reintroduction area with significant stands of lupine. The transect areas are always searched by two spotters. The primary release site is the major transect area. This area has a plot of 120m x 100m with transect widths of 10m. In this primary area a spotter walks every 5m in alternating directions with each other. Secondary transect sites consist of ca.20m wide strips. The route went through the sites, alternating direction from one strip to the next until the entire site was covered. Sightings of KBBs are plotted on a topographic map of the preserve.

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Appendix A

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